

WHAT IS CLAIMED IS:

- 1           1.    A bispecific antibody that binds to:  
2               (a) a first antigen on the surface of effector  
3 cells selected from the group consisting of T-cells and  
4 natural killer cells, and  
5               (b) a second antigen on a 28/32 kDa  
6 heterodimeric protein on the surface of malignant B cells,  
7 which second antigen specifically binds to an antibody  
8 designated 1D10,  
9               wherein the binding of the bispecific antibody  
10 to the first and second antigens results in killing of the  
11 malignant B cells.
- 1           2.    The bispecific antibody of claim 1, wherein the  
2 effector cells are T cells.
- 1           3.    The bispecific antibody of claim 2, wherein the  
2 bispecific antibody binds to a CD3 antigen on the T cells.
- 1           4.    The bispecific antibody of claim 3, wherein the  
2 antibody is produced by the cell line ATCC HB 10993.
- 1           5.    A cell line producing the bispecific antibody of  
2 claim 1.
- 1           6.    The cell line of claim 5 that is a hybrid-  
2 hybridoma formed from two hybridomas,  
3               a first hybridoma producing an antibody that  
4 binds to the first antigen, and  
5               a second hybridoma producing an antibody that  
6 binds to the second antigen.
- 1           7.    The cell line of claim 7, wherein the effector  
2 cells are T-cells and the first hybridoma produces an antibody  
3 that binds to a CD3 antigen on the T-cells.
- 1           8.    A cell line designated ATCC HB 10993.

9. An antibody designated 1D10.

10. A humanized version of the antibody of claim 9.

11. A humanized antibody according to claim 10, the antibody comprising a humanized heavy chain and a humanized light chain:

(1) the humanized light chain comprising three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarity determining regions of the 1D10 immunoglobulin light chain, and a variable region framework from a human kappa light chain variable region framework sequence except in at least one position selected from a first group consisting of L48, L49, L69, and L70 wherein the amino acid position is occupied by the same amino acid present in the equivalent position of the 1D10 immunoglobulin light chain variable region framework; and

(2) the humanized heavy chain comprising three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarity determining regions of 1D10 immunoglobulin heavy chain, and a variable region framework from a human heavy chain variable region framework sequence except in at least one position selected from a second group consisting of H27, H29, H30, H37, H67, H71, H78 and H83, wherein the amino acid position is occupied by the same amino acid present in the equivalent position of the mouse 1D10 immunoglobulin heavy chain variable region framework;

wherein the immunoglobulin specifically binds to a 28/32 kDa heterodimeric protein on the surface of malignant B cells with a binding affinity having a lower limit of about  $10^7$  M<sup>-1</sup> and an upper limit of about five-times the binding affinity of the 1D10 immunoglobulin.

13. The humanized antibody of claim 12, wherein the humanized light chain variable region framework is from the light chain variable region framework of the R3.5H5G antibody except in the at least one position from the first group and except at position L43, which is occupied th amino acid present in the equivalent position of a human kappa subgroup I consensus sequence;

the humanized heavy chain is from the heavy chain region variable framework of the IC4 antibody except in at least one position selected from the second group, and except at position H73, wherein the amino acid position is occupied by the same amino acid present in the equivalent position of a human immunoglobulin subgroup II or IV consensus sequence.

14. The humanized antibody of claim 13, wherein the humanized light chain comprises the amino acid sequence of Fig. 4A (upper) and the humanized heavy chain comprises the amino acid sequence of Fig. 4B (upper).

15. The humanized antibody of claim 14, wherein the humanized light chain further comprises a human kappa constant region, the humanized heavy chain further comprises a human  $\gamma$ 1 constant region, and the humanized antibody effects ADCC and complement-mediated lysis of malignant B-cells when bound to a 28/32 kDa heterodimeric protein on the surface of the cells.

16. A humanized antibody, the antibody comprising a humanized heavy chain and a humanized light chain:

(1) the humanized light chain comprising three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarity determining regions of the mouse M291 immunoglobulin light chain, and a variable region framework from a human kappa light chain variable region framework sequence, and

(2) the humanized heavy chain comprising three complementarity determining regions (CDR1, CDR2 and CDR3)

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having amino acid sequences from the corresponding complementarity determining regions of the mouse M291 immunoglobulin heavy chain, and a variable region framework from a human heavy chain variable region framework sequence except in at least one position selected from a second group consisting of H30, H67, H68, H70, H72 and H74 wherein the amino acid position is occupied by the same amino acid present in the equivalent position of the mouse M291 immunoglobulin heavy chain variable region framework;

wherein the immunoglobulin specifically binds to a CD3 antigen on the surface of T cells with a binding affinity having a lower limit of about  $10^7 \text{ M}^{-1}$  and an upper limit of about five-times the binding affinity of the M291 immunoglobulin.

17. The humanized antibody of claim 16, wherein the humanized light chain variable region framework is from the light chain variable region framework of the HF2-1/17 antibody in subgroup I;

the humanized heavy chain region framework is from the heavy chain region variable framework of the 21/28 antibody except in at least one position selected from the second group, and except at position 44, wherein the amino acid position is occupied by the same amino acid present in the equivalent position of a human immunoglobulin subgroup I consensus sequence.

18. The humanized antibody of claim 17, wherein the humanized light chain comprises the amino acid sequence of Fig. 5A (upper) and the humanized heavy chain comprises the amino acid sequence of Fig. 5B (upper).

19. The bispecific antibody of claim 1 that is humanized.

20. The bispecific antibody of claim 18, wherein the first antigen is the CD3 antigen.

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1           21. The bispecific antibody of claim 20,  
2 comprising:

3           a first binding fragment comprising:

4               a humanized form of the heavy chain variable  
5 region of the M291 antibody;

6               a humanized form of the light chain variable  
7 region of the M291 antibody; and

8           a second binding fragment, which is linked to the  
9 first binding fragment, comprising:

10               a humanized form of the heavy chain variable  
11 region from the 1D10 antibody;

12               a humanized form of the light chain variable  
13 region from the 1D10 antibody;

14               wherein the first binding fragment specifically  
15 binds to the CD3 antigen and the second binding fragment  
16 specifically binds to the 28/32 kDa heterodimeric antigen on  
17 the surface of the malignant B cells.

1           22. The bispecific antibody of claim 21, wherein:

2               the humanized form of the heavy chain variable  
3 region of the M291 antibody comprises three complementarity  
4 determining regions (CDR1, CDR2 and CDR3) having amino acid  
5 sequences from the corresponding complementarity determining  
6 regions of M291 immunoglobulin heavy chain, and a variable  
7 region framework from a human heavy chain variable region  
8 framework sequence except in at least one position selected  
9 from a second group consisting of H30, H67, H68, H70, H72 and  
10 H74 wherein the amino acid position is occupied by the same  
11 amino acid present in the equivalent position of the mouse  
12 M291 immunoglobulin heavy chain variable region framework;

13               the humanized form of the light chain variable  
14 region of the M291 antibody comprises three complementarity  
15 determining regions (CDR1, CDR2 and CDR3) having amino acid  
16 sequences from the corresponding complementarity determining  
17 regions of the M291 immunoglobulin light chain, and a variable  
18 region framework from a human kappa light chain variable  
19 region framework sequence;

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the humanized form of the heavy chain variable region from the 1D10 antibody comprises three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarity determining regions of 1D10 immunoglobulin heavy chain, and a variable region framework from a human heavy chain variable region framework sequence except in at least one position selected from a second group consisting of H27, H29, H30, H37, H67, H71, H78 and H83, wherein the amino acid position is occupied by the same amino acid present in the equivalent position of the mouse 1D10 immunoglobulin heavy chain variable region framework; and

the humanized form of the light chain variable region from the 1D10 antibody comprises three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarity determining regions of the 1D10 immunoglobulin light chain, and a variable region framework from a human kappa light chain variable region framework sequence except in at least one position selected from a first group consisting of L48, L49, L69, and L70 wherein the amino acid position is occupied by the same amino acid present in the equivalent position of the 1D10 immunoglobulin light chain variable region framework.

23. The bispecific antibody of claim 22, wherein the first binding fragment comprises the heavy chain variable region shown in Fig. 5B (upper) and the light chain variable region shown in Fig. 5A (upper), and the second binding fragment comprises the heavy chain variable region shown in Fig. 4B (upper) and the light chain variable region shown in Fig. 4A (upper).

24. The bispecific antibody of claim 23, wherein the first and second binding fragments each further comprises a segment of a constant region fused to the respective heavy chain variable regions, and the binding fragments are linked by association of the constant regions.

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16 second binding fragment specifically binds to the 28/32 kDa  
17 heterodimeric antigen on the surface of th malignant B cells.

1 31. The method of claim 30 further comprising the  
2 step of administering an agent to activate T-cells in the  
3 patient.

1 *21.12.86* *32*  
2 *33.* The method of claim 31, wherein the agent is  
IL-2.

1 33. A pharmaceutical composition comprising the  
2 bispecific antibody of claim 1.

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*add D<sub>4</sub>*